The University of Chicago

THE EARLY DEVELOPMENT OF THE DUCK'S EGG, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE PRIMITIVE STREAK

A PART OF THE DISSERTATION SUBMITTED
TO THE GRADUATE FACULTY IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
BEH KANG CHEN

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THE EARLY DEVELOPMENT OF THE DUCK'S EGG, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE PRIMITIVE STREAK

BEH KANG CHEN

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FOUR PLATES (TWENTY-SIX FIGURES)

AUTHOR'S ABSTRACT

This study deals with the development of the duck embryo from the unincubated blastoderm to the 1-to-3-somite stage. In the unincubated stage the thickened posterior germ wall is the most active region mitotically, contributing cells to complete the entodermal layer. The slightly thickened ectoderm of the posterior half of the pellucid area is the precursor of the embryonic shield. During incubation this ectoderm increases in height, forming the embryonic shield. The primitive streak arises in the posterior part of the shield, where tall ectodermal cells in the midline become several-layered and proliferate cells from their ventral surface which migrate laterally, becoming mesoderm. At first short and broad, the streak becomes longer and narrower until it attains its definitive stage.

The greatest proliferation of ectodermal cells occurs always at the anterior end of the streak of all stages, forming at the definitive stage a definite thickening—Hensen's node. Proliferation diminishes posteriorly along the streak.

The number and orientation of mitotic figures indicate cell movements as follows: Prestreak stages—ectodermal cells migrate from posterior part of shield toward midline, thence forward in midline; early streak stages—anterior movement in streak and to sides of streak, and lateromedial movement from sides toward middle of streak; definitive streak stages—postero-anterior and lateromedial migration of cells.

The embryonic axis, of prestreak and streak stages, lies approximately at right angles to the egg axis in slightly over 50 per cent of the cases.

INTRODUCTION

The early development of the bird egg has been the object of but few investigations, most of which concern the chick. An accurate and complete knowledge of the morphology of these early stages and the mode of formation of the primitive streak from the unincubated blastoderm has become essential for studies in experimental embryology of the bird. A careful and detailed study has therefore been made of the development of the duck embryo from the unincubated blastoderm to the one-to-three-somite stage, including a study of the orientation of mitotic figures at various stages as indicators of the direction of growth and cell movements. The results of this investigation form the subject of the present paper. Pertinent literature will be considered in connection with the various topics.

The problem was suggested by Dr. B. H. Willier, to whom I wish to express my sincere thanks for his advice, suggestions, criticisms, and aid during the progress of the work. I am deeply indebted to Dr. L. H. Hyman for aid in editing the paper.

MATERIAL AND METHODS

White Pekin duck eggs were used, obtained from a farm in Indiana, transported by express to Chicago, requiring about twenty-four hours for transit. The exact time of laying was unknown. The eggs were obtained from March through July and gave a high percentage of hatch. About 14 per cent were sterile and a small number died during incubation. From over 1200 eggs used, 852 embryos were secured, of which 208 were mounted whole, 212 sectioned, and 82 others studied as cleared specimens in wintergreen oil. The remaining embryos have been preserved either in wintergreen oil or embedded in paraffin, but have not been studied.

To insure a uniform rate of development from different lots of eggs, great care was exercised to keep the temperature of the incubator constantly at 39°C. Whenever eggs were put into the incubator, from 300 to 500 cc. of water were added to the sand tray. The time of placing the eggs in the incubator and of removing them was carefully recorded.

The blastoderm was removed as usual under warm salt solution. To aid in separating the vitelline membrane a few drops of a modified Mayer's hardening solution (5 cc. nitric acid, 95 cc. water, two drops glycerin) were added to the blastoderm in a watch-glass. The addition of glycerin to the formula keeps the blastoderm smooth and prevents too great hardening. The vitelline membrane was removed with a wet camel's-hair brush of just a few hairs, and the blastoderm transferred to clear warm salt solution, after which it was fixed for one-half to two hours in Kleinenberg's picro-sulphuric fluid, modified in accord with Lillie's suggestion by the addition of 2.5 to 5 per cent of glacial acetic acid, depending on the size of the embryo. After fixation, the embryos

were washed in weak alcohol and run up to 70 per cent, in which they were preserved.

To study the relation of embryonic axis to the egg axis, the egg was candled and the position of the blastoderm marked on the shell. With the blunt end of the egg to the operator's left, the egg was opened at the marked place, and a piece of agar plate stained with neutral red applied for a few seconds to the vitelline membrane over the blastoderm. The embryo stained by the neutral red is clearly visible, and its angle with the egg axis was then determined with the aid of a protractor in which a triangular opening whose apex pointed toward the 90° mark had been cut. With this opening placed over the blastoderm and the protractor put in line with the egg axis, the deviation of the embryonic axis from the perpendicular could be determined.

Alum-cochineal or Ehrlich's haematoxylin was used for most of the whole mounts and sections. Some serial sections were stained with iron haematoxylin. Mayer's haemalum was advantageous for the study of mitotic figures which were counted under oil immersion. Sections were cut 5 μ and 6 μ in thickness. Transverse sections were prepared of all stages and sagittal sections of many stages in addition. Various measurements were made with an ocular micrometer.

RELATION OF THE EMBRYONIC AXIS TO THE EGG AXIS: BILATERALITY

That a relation exists between the embryonic axis and the egg axis in the hen was discovered by von Baer (1828), who stated that when the blunt end of the egg is held to the observer's left, the embryonic axis lies at right angles to the egg axis with the head of the embryo directed away from the observer. Later investigators found a variable amount of deviation from this rule. Dalton ('81) reported exact agreement with the rule in 50 per cent of cases and Duval ('84) in 76 per cent, for the hen. In the emu Haswell ('87) found that the embryo is usually at right angles, not infrequently oblique, but never parallel, to the egg axis. Féré

('96, '97) recorded a deviation of 45° or more from the perpendicular in 10 to 30 per cent of hen's eggs and in 65 per cent of duck's eggs, the latter bird being therefore more variable than the former. According to Rabaud ('08), the embryonic axis was exactly perpendicular to the egg axis in only nine out of one hundred hen's eggs, but in 76 per cent the axis varied less than 45° to the right and left of the perpendicular. For the pigeon, Patterson ('09) reported that the embryonic axis makes an angle of 45° with the egg axis in 90 per cent of cases, while Bartelmez ('18) found the angle to lie between 45° and 90° in 85 per cent of cases, with variations to almost 180°. The variations are less in eggs from a single bird. The latest data on the hen's egg are those of Kopsch ('27), who found that in only eleven out of 155 eggs was the embryonic axis at right angles to the egg axis, but in fifty cases it was approximately so and ninety-seven, or over 60 per cent, varied 45° or less from the perpendicular in both directions. Kopsch's data agree very well with those of Rabaud, and it may be concluded that for the hen's egg, the embryonic axis is exactly or nearly at right angles to the egg axis in only a small percentage of cases (less than 10 per cent), but varies 45° or less to right and left from the position in 60 to 75 per cent of the embryos.

In the course of my study of the development of the duck, considerable data were accumulated on the orientation of the embryonic axis in unincubated eggs, incubated eggs before the appearance of the primitive streak, and primitive-streak stages. In all cases the blastoderm was rendered visible by applying a neutral-red agar plate to it in situ. In unincubated eggs a deeply stained crescentic area appears at the posterior edge of the area pellucida; in incubated blastoderms in prestreak stages, an ectodermal thickening, the so-called embryonic shield, occupies the posterior half of the pellucid area. A line bisecting these areas was considered to represent the embryonic axis. The data on thirty-one unincubated eggs and 120 eggs incubated one to twelve hours, stages prior to the appearance of the primitive streak, are

summarized in table 1. Of these eggs, the embryonic axis was exactly at right angles to the egg axis in forty-one cases, or 27 per cent. The angle lay within 15° to the right of the perpendicular position in sixteen cases and to the left in twenty-five cases, making a total of eighty-two cases, or over 54 per cent, in which the axis is approximately perpendicular to the egg axis. There were thirty-one additional cases where the axis lay between 15° and 45° to the right of the perpendicular and fourteen to the left. Thus there was a total of 127 embryos out of 151, or over 84 per cent, where the embryonic axis lay within 45° to the right or left of, or on the perpendicular with, the egg axis. This gives a higher percentage of approximate agreement with von Baer's rule for the duck than for the hen. Of the remaining cases, the deviation lay between 0° and 45° in ten cases and 135° and 180° in eleven embryos. There were three inversions where the anterior end of the axis was directed toward the observer when the egg was held in the stated position.

The data for the primitive-streak stages, usually incubated more than nine hours, are presented in table 2. There were 174 embryos, of which the axis lay in the perpendicular in forty-five cases, or approximately 26 per cent. Deviations within 15° of the perpendicular amounted to eighteen to the right, or over 10 per cent, and thirty to the left, or over 17 per cent; the total number approximating the perpendicular is thus ninety-three, or over 53 per cent. Axes lying between 15° and 45° to the right of the perpendicular totaled thirty-one and to the left, sixteen. On adding these forty-seven cases to the preceding, it is seen that 140 cases, or over 80 per cent, of primitive streaks lie within 45° of the perpendicular with the egg axis. The remaining streaks were orientated more than 45° from the perpendicular—seventeen to the right and thirteen to the left—plus four inversions.

The data for the early and late stages are in very good agreement and indicate that the angle of the axis does not alter during development. The agreement with von Baer's rule appears to be somewhat better for the duck than for the

chick. In the duck the embryonic axis was found to lie at right angles to the egg axis in over 26 per cent of cases, as compared with less than 10 per cent in the chick, and to approximate this position in more than half of the specimens, as compared with one-third agreement in the chick. Devia-

TABLE 1

Relation of the embryonic axis to the egg axis before the appearance of the primitive streak

DEGREES	RELATION OF EMBRYONIC AXIS TO EGG AXIS	NUMBER OF EGGS	PERCENTAGE
90	Embryonic axis perpendicular to egg axis	41	27
76–89	Embryonic axis within 15° to the right of the perpendicular	16	11
91–103	Embryonic axis within 15° to the left of the perpendicular	25	16.5
46–55	Embryonic axis within 15° to 45° to the right	5	3.3
56–65	of the perpendicular	8	5.2
66–75		18	12.0
106–115	Embryonic axis within 15° to 45° to the left	12	8.0
116–125	of the perpendicular		
126–135		2	1.3
0–5	Embryonic axis within region from 0° to 45°	1	0.7
10–25	from the horizontal, i.e., the egg axis	3	1.9
26–35		2	1.3
36–45		4	2.6
146–155	Embryonic axis within the region from 135°	4	2.6
156–165	to 180° from the horizontal	3	1.9
166–175		2	1.3
176–180		2	1.3
Inversion		3	1.9
		151	

tions of less than 45° from the perpendicular include over 80 per cent of duck embryos, as contrasted with less than 75 per cent in the chick. The results do not agree with Féré's ('96) findings for the duck and indicate a less rather than a greater variability of the position of the axis in this bird than in the chick. A general impression was gained during the investigation that deviations are more common to the right than to

the left, but the figures lend only a slight support to this conclusion. However, Kopsch's data on the chick show a decided tendency toward right deviation.

This study has shown that regardless of the angle between the embryonic axis, the blastoderm itself is bilaterally sym-

TABLE 2

Relation of embryonic axis to egg axis in primitive-streak stages

DEGREES	RELATION OF EMBRYONIC AXIS TO EGG AXIS	NUMBER OF EGGS	PERCENTAGE
90	Embryonic axis perpendicular to egg axis	45	25.9
76–89	Embryonic axis within 15° to the right of the perpendicular	18	10.3
91–105	Embryonic axis within 15° to the left of the perpendicular	30	17.2
46–55	Embryonic axis within 15° to 45° to the right of the perpendicular	5	2.9
56–65		4	2.3
66–75		22	12.6
106–115	Embryonic axis within 15° to 45° to the left of the perpendicular	7	4.0
116–125		6	3.4
126–135		3	1.7
0–5	Embryonic axis within region from 0° to 45° from the horizontal, i.e., the egg axis	3	1.7
10–25		5	2.9
26–35		4	2.3
36–45		5	2.9
136–145	Embryonic axis within the region from 135° to 180° from the horizontal	2	1.2
146–155		2	1.2
166–175		4	2.3
176–180		5	2.9
Inversion		4	2.3
		174	

metrical even before incubation. The crescentic area which is seen after staining at the posterior edge of the area pellucida is bilateral and a line bisecting it marks the future axis of the embryo. Bilaterality is thus fully established before the organization of the primitive streak, which forms upon an already existing axis of symmetry and does not itself determine the axiation or bilaterality of the embryo.

STRUCTURE OF THE UNINCUBATED BLASTODERM

The unincubated blastoderm of the chick has been repeatedly described in the older literature, but here it will suffice to combine Lillie's ('19) and Wetzel's ('29) descriptions for comparison with the duck. The unincubated blastoderm of the hen's egg averages 4.4 mm. in diameter (Edwards, '02), of which 1.75 to 2.5 mm. is occupied by the central transparent area pellucida (Keimfeld of Wetzel), the remainder by the darker peripheral area opaca. Between the area pellucida and the yolk is the subgerminal cavity (subgerminale Höhle or Dotterhöhle of Wetzel), bounded peripherally by the germ wall, a thick layer of cells continuous with the yolk. The germ wall is frequently syncytial and filled with yolk inclusions. It is thicker at the future posterior end of the blastoderm than elsewhere. The area pellucida consists of an outer ectoderm, a single layer of cylindrical cells, and an inner incomplete entoderm. The ectoderm is continuous peripherally with the germ wall. The entoderm underlies only the posterior part of the area pellucida and is there continuous with the thickened germ wall. Anteriorly and laterally the entoderm does not reach the germ wall, but terminates with irregular edges. The ectoderm is thickened in the central region of the area pellucida, constituting the embryonic shield. The entoderm, elsewhere one-layered, is several-layered centrally, forming a thickening called by Wetzel the Entodermhof.

The unincubated blastoderm of the duck has not been hitherto described; it corresponds closely to that of the chick. In stained whole mounts it is seen as a circular area divisible into the central transparent area pellucida and the opaque peripheral area opaca. Immediately bounding the area pellucida is the germ wall, notably thicker and wider at the future posterior end of the blastoderm. The diameters of thirty-seven unincubated blastoderms mounted in balsam or oil of wintergreen ranged from 1.8 to 2.8 mm., with an average of 2.24 mm.; those of the area pellucida, from 0.83 mm. to 1.5 mm., with an average of 1.14 mm. Thus the duck ger-

minal disk is smaller than that of the chick despite the larger size of the egg. Figure 1 is a photograph of the unincubated blastoderm of the duck.

Study of sections of twelve unincubated blastoderms shows that in all cases they are composed of two layers, ectoderm and entoderm. The ectoderm is a single coherent layer of epithelial cells throughout the areas pellucida and opaca. In the posterior half of the pellucid area, the ectoderm is slightly thicker than elsewhere, composed of tall, cylindrical cells (average height, 25 μ), with irregularly arranged nuclei, not lying in one level. The thickened ectoderm is probably the precursor of the embryonic shield. Elsewhere in the area pellucida the ectoderm is lower, of somewhat hexagonal cells. It extends out over the area opaca as a still more flattened membrane, reaching beyond the germ wall, particularly posteriorly and laterally. The ectoderm cells have definite cell walls, distinct round nuclei, and a moderate number of yolk granules.

The entoderm underlies the whole area pellucida, but is not a coherent layer. In the posterior part of the area pellucida it is several layers thick and is closely united with the germ wall. Anteriorly from this region, the entoderm thins rapidly and is soon reduced to a one-celled condition, where, however, the cells are very loose and incoherent. In fact, in the anterior part of the area pellucida, the entoderm consists of scattered single cells, with here and there groups of a few cells. The entoderm cells are spherical and contain numerous yolk inclusions; and many yolk bodies are intermingled with them. The entoderm touches the germ wall throughout the entire circumference of the germ wall, but is closely related to it only posteriorly.

Encircling the area pellucida is a thick zone which is wider and thicker posteriorly than elsewhere. This zone I have taken to include the germ wall, zone of junction, and margin of overgrowth of Lillie's ('19) and Patterson's ('09) descriptions. It is not possible in the duck to distinguish between a central cellular part of the germ wall and a peripheral syncy-

tium (zone of junction). Consequently the entire thickening will be designated germ wall. It is almost twice as thick at the posterior as at the anterior border of the area pellucida. The average thickness for twelve cases is 83.5 μ posteriorly, 42 to 30 μ at the sides, and 42 μ anteriorly. The germ wall consists of large cells without distinct boundaries and hence forming a syncytium. This syncytium is packed with yolk spherules, especially where it borders the entoderm, and is continuous below with the yolk. Peripherally beyond the thickened germ wall, the ectoderm extends out over the free yolk as a flattened epithelium, distinct from the yolk. This may correspond to the margin of overgrowth of Lillie and Patterson, but does not quite fit their figures and definition. In nine of the twelve blastoderms, this free ectodermal margin is well developed; in the other three the ectoderm does not extend beyond the germ wall thickening.

A median sagittal section through the unincubated blastoderm is shown in figure 9. Each end of the section is composed of the thick germ wall, which is much thicker and broader at the posterior end. It begins posteriorly as a pointed projection of rather well-defined epithelial cells, which may be termed the margin of overgrowth. Just anterior to this is the thick germ wall proper, whose upper layers are epithelial with distinct cell walls, lower layers more or less syncytial with numerous yolk inclusions and intermingled with free yolk. Anterior to the germ wall is the area pellucida, consisting of a continuous ectoderm above, composed of rather irregularly shaped and arranged cells, and an incoherent entoderm below of rounded, separate yolk-laden cells. The entoderm is three or four layers thick immediately in front of the posterior germ wall, with which it is continuous. Anteriorly it thins to a single layer of separate cells which here and there may cohere for a short distance. The ectoderm of the posterior half of the area pellucida is thicker than that of the anterior half. The anterior germ wall is similar to the posterior, but is less thick. The entoderm reaches the anterior germ wall. In this particular

blastoderm the free marginal overgrowth of the ectoderm mentioned above is lacking.

The number and orientation of mitotic figures were carefully studied in sections of nine unincubated blastoderms. Three orientations were considered: dorsoventral spindles, oriented at right angles to the surface; anteroposterior spindles, oriented parallel to the surface, but lying on lines parallel to the anteroposterior axis of the blastoderm; and lateral spindles, also horizontal and parallel to the surface, but lying on lines crossing the blastoderm at right angles to its anteroposterior axis. In each blastoderm the mitotic figures were counted in one section each of three different levels. For the unincubated blastoderm the levels chosen were the posterior thickening of the germ wall, the posterior part of the area pellucida, and the central part of the area pellucida. But one section was counted for each blastoderm and those of the same level were added together for the nine blastoderms. The figures given are thus the sum of the mitoses found in single corresponding sections of nine blastoderms. For the posterior thickening of the germ wall, the entire count thus obtained for the nine blastoderms, one section of each, was fifty-four dorsoventral spindles, sixty-one anteroposterior, and twenty-three lateral spindles. It is assumed that the dorsoventral spindles are adding cells to the thickness of the germ wall, the anteroposterior spindles proliferating cells into the entoderm, and the third class helping increase the circumference of the germ wall. In cross-sections of the posterior part of the area pellucida, including laterally the germ wall, were counted a total of eighteen dorsoventral, forty-two anteroposterior, and twenty-eight lateral mitotic figures in the ectoderm; for the entoderm the figures are eleven, twenty-six, and six, respectively. Central levels of the area pellucida including the germ wall gave for the ectoderm fourteen dorsoventral figures, thirty-eight anteroposterior, and six lateral ones; for the entoderm, three, twenty, and seven spindles, respectively.

These figures indicate that the posterior germ wall is the region of most active cell proliferation of the blastoderm and that it is increasing in thickness and adding cells anteriorly into the entoderm. The ectoderm of the pellucid area is spreading about equally in all directions by means of divisions both perpendicular and parallel to the surface. Since the ectoderm remains one-layered, it may be assumed that the cell products of vertically oriented spindles work into the ectoderm by rearrangement. The entoderm is less active than the ectoderm and is advancing more anteriorly than in other directions.

It should be remarked that the majority of the mitotic figures were in the metaphase. Since mitosis under the action of inhibiting conditions tends to pause in the metaphase, this suggests that the figures found were those present at the time of laying and not new mitoses initiated at room temperature. The fall of temperature after laying probably caused the early mitoses present to slow down and halt at the metaphase.

This study of the cellular disposition and mitotic frequency in unincubated blastoderms suggests strongly that the entoderm is advancing forward toward the anterior end of the area pellucida by proliferation from behind, particularly from the posterior germ wall. There was no evidence of an invagination in the posterior germ wall nor is any blastoporal split present, but the observations are not inconsistent with Patterson's account of the formation of the entoderm in the pigeon by a turning under at the posterior margin of the blastoderm (Patterson, '09). To determine the mode of origin of the entoderm in the duck, stages prior to laying must be studied.

FROM THE FIRST TO THE TWELFTH HOUR OF INCUBATION

Neither Lillie nor Wetzel discussed the early incubation stages of the chick to any extent. Duval's ('89) figures of five- and seven-hour blastoderms appear to be erroneous, for the embryonic shield is lacking and the blastoderm is shown

separated by clefts from the surrounding yolk. Mitrophanow's ('99) account of the chick appears to be correct and is here briefly abstracted. The germinal disk increases from a diameter of 3 to one of 4.5 to 6 mm., the area pellucida from 2 to 2.3 to 3 mm., by the eighth to ninth hour of incubation, at which time the primitive streak begins to appear. The thicker central ectoderm continues to thicken, reaching a height of 30 to 35 μ before the appearance of the streak. The ectoderm separates from the entoderm, beginning in the center and extending to the periphery. Schauinsland ('03) has figured a sparrow embryo in a prestreak stage. The section shows the central thickening of the ectoderm and a complete entoderm in the area pellucida.

Mitrophanow ('02) has described early incubation stages of the duck: five and one-half, six and one-half, nine, ten, and eleven hours. At five and one-half hours, the embryonic shield was present, 32 μ in height at its center. By six and one-half hours this had reached a maximum of 43 μ and was composed of clear cylindrical cells which had lost their yolk inclusions; the entoderm formed a continuous layer separated centrally from the ectoderm. At ten hours, the separation of ectoderm and entoderm was complete throughout the whole pellucid area. The primitive streak appears at the eleventh hour. My observations are similar to those of Mitrophanow, but are based on more complete series and more abundant material.

In general, the early development of the duck proceeds more slowly than that of the chick. The ectodermal shield of the duck is thicker, the entoderm does not become a coherent layer until ten to twelve hours of incubation, and the primitive streak appears at about twelve hours as contrasted with eight hours in the chick.

1. One- to three-hour stages

Twenty-four whole mounts and sixteen sectioned blastoderms of these stages were studied. There is practically no change from the unincubated state. The diameter of blasto-

derm and pellucid area have not altered. There is a slight diminution in the thickness of the germ wall, which averages 75.2 μ posteriorly and 39.3 μ anteriorly. Ectoderm and entoderm cells of the various regions practically correspond in height and extent to those of unincubated blastoderms.

2. Four to six hours

Study of twenty-four whole mounts and twenty-one sectioned series shows an increase in the diameter of the area pellucida and in the width of the area opaca. The former averages 1.32 mm.; the latter, 0.57 mm. The thickness of the posterior germ wall is again diminished, averaging 55 μ as compared with 83.5 μ of unincubated blastoderms. The thickened ectoderm of the middle posterior region of the pellucid area (embryonic shield) is higher than before, averaging 27 μ as compared with 25 μ of previous stages. In whole mounts the posterior half of the area pellucida appears a little darker, probably because of the thickened ectoderm of the embryonic shield and forward extension of the entoderm. The condition of the entoderm and the germ wall is the same as in the stage next to be described.

3. Seven to nine hours

The blastoderm has continued to increase in diameter, the area pellucida averaging 1.37 mm. in diameter, while the area opaca is 0.59 mm. wide. Study of twenty-one whole mounts shows that the dark area mentioned above has become larger and more conspicuous. It now occupies the posterior half of the area pellucida, presenting a convex anterior border rather sharply defined from the lighter-staining anterior part of the pellucida area. This darker-staining area is called the embryonic shield by many of the older writers. The name seems to have originated with von Baer. The darker appearance of this area is probably caused by the greater height of ectodermal cells and the greater thickness of the entoderm in this region. Examination of twenty sectioned blastoderms reveals but little change in the ectoderm from preceding stages.

The entoderm completely underlies the area pellucida and is much more coherent than in earlier stages, although still presenting gaps between the cells. It is several-layered in the posterior third of the area pellucida and thins anteriorly to a single loose sheet. The impression is gained that entodermal cells have migrated forward from the posterior thickening to fill the gaps in the entoderm. The germ wall is similar to the unincubated stage, thicker posteriorly than elsewhere, more or less syncytial, and mingled with yolk spherules. Beyond the germ wall, the free ectoderm extends farther than before as a rather flat membrane overlying the yolk.

4. Ten to twelve hours

The diameter of the pellucid area has increased to 1.63 mm. and the width of the area opaca to 0.78 mm. Twenty whole mounts and twenty-three sectioned series of this age were prepared. Figure 2 is a photograph of a blastoderm of this age. As compared with the unincubated blastoderm (fig. 1), the distinction between area pellucida and area opaca is now evident. In the area pellucida the noteworthy feature is the circular dark area in the posterior central part. This is the embryonic shield or ectodermal thickening. It seems to have been mistaken by some workers for the earliest appearance of the primitive streak.

Sections show the embryonic shield to be composed of a thickened ectoderm which in its central portions is increased in height (28.9 μ) over preceding stages. The cells are tallest behind the center of the area pellucida and in front of the posterior germ wall and diminish toward the periphery. The cells of the shield are tending to become pseudostratified. The extent of the shield has enlarged over previous stages. The thickness of the posterior germ wall has remained about the same since the four-to-six-hour stage, about 55 μ. The entoderm now consists of a practically continuous layer of spindle-shaped cells. It is several layers thick posteriorly, single-layered anteriorly. Whereas in previous stages the entoderm cells were spherical, yolk-laden, and not in contin-

uity, they are now more flattened, less yolk-filled, somewhat spindle-like, and constitute a continuous layer.

Figure 10 is a drawing of a median sagittal section of a blastoderm of ten hours of incubation. It illustrates the points described in the preceding paragraph: the thickened posterior germ wall, the thick pseudostratified ectoderm of the embryonic shield, and the continuous entoderm layer, thickened posteriorly beneath the embryonic shield.

5. Abundance and distribution of mitotic figures

Counts have been made of the mitotic figures in the embryonic shield and underlying entoderm to throw some light on cell movements during prestreak stages. Posterior, middle, and anterior levels of the shield were studied in nine blastoderms. The anterior level lies at about the center of the area pellucida; the posterior, just in front of the thickened germ wall. As before, one section of each level was studied in each blastoderm and the mitotic figures found summed for the corresponding levels of the nine blastoderms. In the ectoderm there were counted a total in the nine specimens of three dorsoventral, forty-four anteroposterior, and forty-one lateral spindles in the posterior level; three, fifty-three, and twenty-seven, respectively, in the middle; and none, thirty-seven, and nineteen, in the anterior level. The preponderance of anteroposterior spindles indicates that cells are moving forward in the embryonic shield and the large number of lateral spindles suggests a spreading of the blastoderm peripherally and an addition of cells toward the center. It may be concluded that cells are moving from the periphery toward the center of the embryonic shield and forward in the shield. In the entoderm the figures were: for posterior levels, four dorsoventral, twelve anteroposterior, and four lateral spindles; for the middle levels, none, fifteen, and four, respectively; and for the anterior levels, none, five, and six, respectively. It is seen that the entoderm is not very active, for the number of mitotic figures has decreased since the unincubated stage. The majority of its figures suggest a forward

migration of cells from the posterior thickened region to fill the gaps in the more anterior levels of the entoderm.

PRIMITIVE-STREAK STAGES

That the bird embryo takes origin from a linear darkening in the midline of the area pellucida was known to the earliest investigators of embryology. Since the time of von Baer, this darkening has been called the primitive streak, after his designation. Waldeyer ('69) called attention to the fact, which he states had been overlooked by previous investigators, although represented by His ('68) in figures, that at its first appearance the streak is shorter, broader, and less definite than later. This early streak he designated the broad (breite) primitive streak; the later narrower, more elongated, and sharply defined structure, the narrow (schlank) streak. Mitrophanow ('99) has given a number of drawings and photographs of broad and narrow streaks of the chick. Wetzel ('29) gives a series of drawings of whole mounts and sections of various stages in the growth of the streak of the chick. According to him, and also Mitrophanow, the streak appears at about the eighth hour of incubation as a somewhat conical projection extending from the thickest region of the posterior germ wall forward along the median line of the circular area pellucida. This short primitive streak, as Wetzel designates it, at first extends only one-fifth to one-fourth of the diameter of the still circular pellucid area, but soon reaches half the distance and then presents a thickened anterior end, the primitive knot or Hensen's node, and behind this the primitive groove. By about the twelfth hour of incubation, the pellucid area has begun to elongate posteriorly into the typical pyriform shape, and primitive knot and groove are more pronounced. By sixteen to eighteen hours, the definitive narrow elongated streak has developed along the midline of the pyriform area pellucida, which is 3 to 3.5 mm. long. At the anterior end of the streak the primitive knot forms a thick, sharply bounded swelling. Hensen's node bearing a central depression, the primitive pit, from which the primitive

groove, bounded by the primitive folds, extends posteriorly and terminates near the posterior margin of the area pellucida, usually by a forked end. Dimensions are 1 mm. or a little less for the short primitive streak to 2 to 2.5 mm. for the definitive streak.

Primitive streaks of other birds—albatross, sparrow, starling—are illustrated by Schauinsland ('03). These resemble that of the chick, starting out as a short broad thickening which gradually elongates and narrows. The streak of the sparrow differs from that of other birds in that the rear end of early stages lies well within the area pellucida and does not make contact with the posterior germ wall. All subsequent workers except Duval are agreed that Koller's ('82) idea of the origin of the streak from a sickle-shaped thickening in the posterior germ wall is incorrect. Mitrophanow ('02) illustrates the primitive streak of a tern (Sterna hirundo).

The only work upon the early primitive streak of the duck is that of Mitrophanow ('02). He found the primitive streak appearing at ten or eleven hours of incubation, first as a darkened area in front of the posterior germ wall and later extending back to the germ wall. Later stages showed more elongated streaks, scarcely, however, narrower.

The primitive streak, as known to early observers, is a median ectodermal thickening in the posterior half of the area pellucida. It involves the thickened ectoderm of the previously existing embryonic shield, but otherwise bears no relation to the shield (Waldeyer, Mitrophanow). Mitrophanow ('99, '02), after careful studies of the earliest recognizable stages of the streak in duck and chick, states positively that the ectodermal proliferation begins at or a little behind the center of the area pellucida and at first does not reach the thick posterior germ wall. This is at variance with Wetzel's account, which is probably the most accurate. According to him, the ectoderm, previously one-layered, becomes several-layered in the streak, showing numerous mitotic figures above, and projecting below irregularly toward the

entoderm. The streak is widest posteriorly, but the thickening is deeper anteriorly (primitive knot). Cells proliferate ventrally from the ectoderm and fill the space previously present between ectoderm and entoderm. Wetzel's figures indicate, although this is not clearly stated in the text, that the proliferating band of ectoderm extends the length of the short primitive streak to the germ wall. The streak early becomes divisible into three regions: the primitive knot or Hensen's node, a thick proliferating region at the anterior end of the streak, closely bound to the entoderm, and containing the deep primitive pit; the Ebene, or less thick, looser region beyond the node, loosely connected to the entoderm and with a shallow, flat, primitive groove; and the broad, flat rear end of the streak, again bound to the entoderm and with a deep groove. In reporting my observations on the primitive streak of the duck, I shall divide the material into four arbitrarily selected stages.

1. Short primitive streak (Wetzel)

In the description of prestreak stages it was emphasized that the ectoderm of the central and posterior part of the area pellucida is thickened to form the embryonic shield. The height of the ectodermal cells of this shield increases during incubation until by ten to twelve hours the tallest cells are nearly 30 μ in height. They tend to be pseudostratified. They lie behind the middle of the area pellucida and in front of the thickened germ wall. The primitive streak of the duck begins as a thickening and proliferation of this tall ectoderm of the embryonic shield.

Fifteen blastoderms in the earliest visible or short primitive-streak stage were studied. They were examined and sketched whole, and twelve were sectioned. The average diameter of the blastoderms was 3.4 mm.; of the area pellucida, 1.67 mm.; the length of the short primitive streak averaged 0.32 mm., or about one-fifth the length of the area pellucida. The time of incubation varied from nine to fifteen hours.

The first appearance of the primitive streak in the duck may occur at about nine hours, but usually is evidenced from the twelfth to the fifteenth hours of incubation. These observations accord with those of Mitrophanow ('02). Figure 3 is a photograph of the earliest visible or short primitive streak of the duck. The streak appears as a short, vaguely outlined, hemi-elliptical darkening in the posterior central part of the area pellucida. In some cases, as in the figure, the streak projects forward from the posterior germ wall; in other cases it does not in the whole mount appear to be connected with the germ wall. The area pellucida is still quite circular and scarcely enlarged since the last stages considered; but the blastoderm as a whole has enlarged, due to an increase in the width of the area opaca.

Sections through these blastoderms show that the primitive streak consists of a thickened and proliferating ectoderm. The ectoderm is thickest at the anterior end of the streak (average of 40 μ) and diminishes posteriorly to 28.4 μ just in front of the germ wall. The streak is, however, broader posteriorly; the measurements of its width are 280 μ at the posterior end and 189 μ at the anterior end. Proliferation occurs along the entire length of the streak to the germ wall.

Sections of these early streaks present the following picture (fig. 12). At the anterior end of the streak the ectoderm becomes rather abruptly several-layered in the center of the blastoderm and forms a mass projecting ventrally toward the entoderm. The ectoderm cells of this mass are no longer of columnar form as previously, but polygonal. In the dorsal part of the mass the cells are compact and in close contact with each other; ventrally they become looser and more rounded and touch the entoderm. There is as yet no lateral migration of the cells as mesoderm. Outside of the streak the ectoderm is thickened and pseudostratified from the anterior part of the area pellucida back around the anterior two-thirds of the streak and laterally to the margin of the area pellucida. This thickened ectoderm, which can sometimes be seen in whole mounts as a slightly dark area, corre-

sponds to the Scheibe of Wetzel. It is considered by him the prospective medullary plate.

In two cases out of eleven a very shallow primitive groove was seen in the anterior part of the streak. It is shown in figure 12.

The entoderm is a coherent layer chiefly one cell thick. The posterior thickening is less marked than previously, but here the entoderm is still three or four layers thick. Elsewhere it is more or less one-layered with additional cells here and there (fig. 12). Anterior to the streak (fig. 11), the entoderm is thinner and less yolk-laden. There was no evidence of a definite Entodermhof (Wetzel) in front of the streak, where in the chick patches several cells thick occur. The entoderm of the duck may exhibit such patches of additional entoderm cells anywhere throughout the area pellucida. The entoderm is thicker posteriorly as in earlier stages.

The germ wall is much the same as previously except that it has extended considerably peripherally so that the thin margin of overgrowth is much wider than before. It is two or three cells thick and terminates with a pointed margin.

Figure 11 is a section through the blastoderm anterior to the streak showing the thickened ectoderm of the Scheibe and the continuous entoderm with occasional extra cells. Figure 12 passes through the center of the streak and illustrates the proliferating median region, the very thick ectoderm of the Scheibe, and the shallow primitive groove.

2. Medium primitive streak

This name is applied to stages in which the streak is longer than the preceding, about twice as long, but in which the area pellucida still preserves its circular contour. Rabl presents one embryo of this stage (his fig. 3), incubated twenty-four hours; length of the streak, 0.64 mm. The length agrees with my embryos of this stage, but the time of incubation is shorter in my cases. Rabl records that sections of this embryo revealed a very shallow primitive groove in the anterior part of the streak and a head process; the embryo

is thus more advanced than mine of the same length. I examined twenty-one embryos, seven as whole mounts, fourteen as serial sections. The average diameter of the blastoderm was 3.8 mm.; of the area pellucida, 1.68 mm.; the width of the area opaca averaged 1.06 mm. The streak (fig. 4) appears as an elongated club-like darkening in the center of the posterior half of the area pellucida. Its length averaged 0.61 mm., and it extends a third to a half of the diameter of the area pellucida. Around the anterior part of the streak there may appear a darker area probably representing the Scheibe. These stages were obtained during thirteen to twenty-one hours of incubation.

Sections reveal a picture similar to the preceding stage except that the proliferative thickening of the ectoderm occupies a longer area. Figures 13 to 15 illustrate sections anterior to the streak, through the anterior end of the streak and through its posterior end. The anterior end of the streak is 182 μ wide and 43 μ thick; the posterior end, 261 μ wide and 27.7 μ thick. As in the earlier stage, the posterior end is wider, but the thickening is deeper anteriorly. The main part of the streak (fig. 14) consists of a several-layered ectoderm bulging ventrally. The lower layers are looser and extend to the entoderm and some of the cells have become free as mesoderm. Anterior to the front end and continuous with the streak occurs a group of mesoderm cells proliferated from the ventral side and anterior end of the streak (not illustrated in the figures). These are called the anterior free mesoderm by Wetzel and the preaxial mesoderm by Adelmann ('22). The ectoderm anterior to the streak (fig. 13) and around two-thirds of the streak (fig. 14) is thickened as the Scheibe. It presents the appearance of a pseudostratified epithelium. It is thickest at the sides of the streak (31 μ). It extends nearly to the margin of the area pellucida, where the ectoderm becomes a thinner ordinary epithelium (18.2 μ) and extends out over the area opaca as a flat membrane.

A shallow primitive groove was seen in three of the fourteen specimens in the anterior part of the streak. The con-

dition of the entoderm and germ wall is practically unchanged. The figures (13 to 15) illustrate their appearance.

3. Mitotic figures in early streaks

The distribution and orientation of mitotic figures in these two early stages of the streak were studied to ascertain possible cell movements. In serial sections of thirteen embryos examined under oil immersion mitotic figures were found in all levels of the streak and in regions immediately lateral to the streak. There were 115 spindles in anterior levels, seventy-one in middle levels, and sixty-eight posteriorly. This suggests that the anterior end of the streak is the most active proliferating region and that this activity decreases posteriorly (compare Hyman, '27). Further analysis of the spindles shows that there are sixty-seven anteroposterior spindles, thirty-two lateral spindles, and one dorsoventral spindle in a section through the anterior level of the streak. The majority (twenty-five) of the lateral spindles and also most (forty-nine) of the anteroposterior spindles are in the sides rather than in the main part of the streak. These data indicate a proliferation forward from the anterior part of the streak and from the sides toward the middle as well as forward. In the middle level of the streaks were counted a total of forty anteroposterior spindles, twenty-seven lateral, and six dorsoventral. Again the majority of the first two sorts lay in the sides of the streak. Posterior levels gave counts of forty-three anteroposterior spindles, sixteen lateral ones, and nine dorsoventral ones. Here the majority of the anteroposterior spindles were in the area pellucida, indicating forward movements here. Altogether the data suggest anterior movements in the streak and to the sides of the streak and lateromedial movements from the sides toward the middle of the streak. These findings are in accord with the conclusions of Wetzel and Gräper which will be discussed later.

4. Elongated primitive streak

This name is applied to rather elongated streak stages in which the area pellucida has begun to elongate posteriorly and assume the familiar pear shape. It somewhat corresponds to the broad primitive streak of Waldeyer ('69, fig. 9). Wetzel presents a good figure (fig. 14) of the chick of this same stage, and Rabl ('23) shows three duck embryos of corresponding appearance (figs. 4, 5, 6). These have pyriform pellucida areas with elongated streaks, averaging 0.9 mm. in length, which project from the posterior germ wall forward along the midline of the area pellucida. The streaks are surrounded by a faint circular area which Rabl regards as indicating the limits of the mesoderm. In whole mounts no primitive groove appeared, but sections showed that one was present in the three specimens of this age which Rabl studied.

My material of this stage comprises eighteen sectioned embryos and eleven whole mounts. They were obtained between thirteen and twenty-one hours of incubation. In a few cases this stage was seen as late as twenty-three to twenty-five hours. The average diameter of the blastoderm was 4.2 mm. The average length of the pear-shaped area pellucida was 1.8 mm. The streaks found in such pellucid areas varied in length from 0.45 to 1.03 mm., with an average of 0.9. Two-thirds of them were more than 0.7 mm. long.

In whole mounts (fig. 5) the area pellucida is rounded in front and narrowed posteriorly, presenting a pyriform or conical shape. The streak extends from the posterior bay of the area pellucida forward to about its center as a darker-staining bar. Its posterior end is wider and thinner than the anterior end. The anterior end, although darkly stained and rather definitely outlined, can scarcely be designated as Hensen's node at this stage. No primitive groove is apparent in whole mounts, although sections reveal its presence. The posterior part of the streak is more vague and less deeply stained than its anterior portion. The Scheibe is faintly evident as a darker area around and in front of the anterior part of the streak.

A series of sections through a primitive streak of this age is presented in figures 16 to 19. Figure 16 is taken just in front of the anterior end of the streak. It shows the thickened ectoderm of the Scheibe, extending to the margins of the area pellucida. This ectoderm has the appearance of a pseudostratified epithelium; it is composed of elongated cells with nuclei arranged in two levels. Below the central part of the Scheibe is seen the loose mass of the preaxial mesoderm. Figure 17 passes through the anterior end of the streak or Hensen's node, not as yet very sharply defined. The streak is here a narrow, well-delimited band of thickened ectoderm eight or nine cells thick, bulging ventrally to touch the entoderm. Its thickness here, including the attached mesoderm, varies from 24 to 56 μ, with an average of 49 μ; its width averages 173 μ. The mesoderm forms the thick lower part of the streak; a few free mesoderm cells are beginning to wander laterally from the central mass. The Scheibe reaches the margin of the area pellucida as before. A section of the rear end of the streak is shown in figure 18; its thickness here is decreased to 20 to 32 μ, with an average of 23 μ; its width averaged 300 μ. The streak is thus narrow, but deep anteriorly and wide and shallow posteriorly. The posterior thinning of the streak may be related to the backward extension of the margin of the area pellucida which carries the streak with it. At this level (fig. 13), the Scheibe no longer is present, the ectoderm outside the streak being an ordinary epithelium. Below the ectoderm are a few mesoderm cells. The entoderm throughout these sections is a thin coherent layer of flattened, spindle-shaped cells, with here and there extra cells above the regular layer. There is no definite Entodermhof, that is, region of thickened entoderm, anterior to the streak, as noted by Wetzel for the chick. At the posterior end of the streak (fig. 18), the entoderm is still thick as in early stages, consisting of several layers of yolk-laden cells. This passes directly into the thickened posterior germ wall, shown in figure 19, through the extreme posterior end of the streak, which is thus seen to overlie the germ wall.

The germ wall is about as previously, thick posteriorly, thinner elsewhere. The mesoderm has begun to migrate laterally from the primitive streak. In most of the cases (ten out of eighteen) there are a few scattered cells beginning to move laterally from the streak all along the streak. In two cases, the mesoderm has reached the area opaca in posterior levels of the streak. In the other six cases there is scarcely any migration of mesoderm.

The primitive groove cannot be seen in whole mounts, but the sections reveal its presence in the majority of cases as a very broad shallow depression, slightly deeper anteriorly. Of the eighteen sectioned embryos, ten showed a shallow groove, three gave some slight evidence of a groove, and in five the groove had not yet formed.

The mitotic figures were studied in thirteen streaks of this age and the total count was: 102 spindles in sections of anterior levels, ninety-eight in sections of the middle regions, and sixty-five in posterior sections. Again, the greatest proliferative activity occurs in anterior levels. When analyzed as to orientation of spindles, the data yield forty-six anteroposterior spindles, forty-six lateral spindles, and six dorsoventral spindles in anterior levels. Again the great majority of the anteroposterior and lateral spindles lay in the sides of the streak rather than in its center. This indicates not only general forward growth, but proliferation of cells from the sides into the axis of the streak. In middle levels, there were thirty-seven anteroposterior spindles, fifty-four lateral, and six dorsoventral. The preponderance of lateral spindles suggests marked movement from the sides into the center in middle levels of the streak. Posteriorly the mitotic actvity is lessened, with twenty-nine anteroposterior spindles, twenty-nine lateral ones, and five dorsoventral. All levels show postero-anterior and lateromedial movements of cells.

5. Definitive primitive streak

This stage of the narrow, fully developed streak has been made familiar in the textbooks of embryology. Duval figures

it for the chick, Schauinsland for the sparrow and albatross, and Rabl for the duck. According to Wetzel, this stage is reached in the chick in sixteen to twenty hours of incubation, while Duval gives thirteen to sixteen hours. In the duck, Rabl presents similar stages obtained at twenty-nine to thirty hours of incubation. Schauinsland does not give the times of incubation. The primitive streak is similar in these various birds, consisting of a narrow, elongated rod-like body lying along the midline of the pellucid area. Its anterior end is thickened to form Hensen's node, and behind this the streak consists of the primitive groove bounded on each side by the primitive folds. The posterior end may be forked and, according to Wetzel, usually is so in the chick. In the chick, according to Wetzel, the definitive streak is about 2.5 mm. long, while the length of the area pellucida at this time is 3 to 3.5 mm. Rabl's average figures for his two duck embryos of this stage are 1.6 mm. for the pellucid area and 1.3 mm. for the streak. These figures agree very well with mine, but only one of Rabl's figures (fig. 6) presents a typical appearance with a fully developed groove. As in the other stages of development, the duck embryo is smaller than the chick.

In the duck, according to my results, the definitive streak is attained at eighteen to twenty-five hours of incubation. In twenty-two whole mounts the average diameter of the blastoderm was 5.70 mm. The area pellucida is decidedly pyriform, broad in front, narrow behind, with an average length of the area pellucida ranging from 1.4 to 2.7 mm.

The streak (fig. 6) appears as an elongated darkly stained rod extending from the posterior part of the area pellucida along the midline of the area. It occupies about three-fifths of the length of the area pellucida and averaged 1.2 mm. in length, with variations from 0.8 to 1.5. Its anterior end is thickened and deeply stained, forming the primitive knot or Hensen's node. Behind this is seen a clear streak, the primitive groove, bounded laterally by the primitive folds. The primitive pit is not evident nor is Hensen's node as well developed as in the chick. The posterior part of the streak

is often faint and often cannot be traced in whole mounts to the rear margin of the pellucid area. In some streaks whose length puts them into this stage, the primitive groove is not visible in whole mounts. Of the twenty-two specimens, the groove was deep in nine, shallow in six, and lacking in seven. Two showed no Hensen's node, either. The Scheibe is detectable in some specimens as a slightly darker area around the anterior third of the streak. The posterior end in the duck is never forked as seems to be commonly the case in the chick; but it is frequently curved or bent to one side. Of twenty-five embryos, the rear end of the streak was curved to the right in thirteen cases, to the left in six, and was straight in six.

Sections of eighteen embryos are available; figures 20 to 24 give various levels of a typical definitive streak with definite node and groove. Sections through the anterior part of the area pellucida show complete ectoderm and entoderm layers separated from each other. There is no mesoderm. The ectoderm here consists of a moderately tall epithelium, one-layered, slightly thickened toward the center and diminishing laterally to the edge of the area pellucida, where it becomes a thin membrane over the yolk. The entoderm is a continuous, somewhat flattened epithelial layer, with additional cells here and there. At the margins of the area pellucida, the entoderm becomes continuous with the germ wall. A little posteriorly, in sections shortly anterior to Hensen's node (fig. 20), the central ectoderm is seen to be much thickened and presents the pseudostratified appearance previously described, constituting the Scheibe, which extends nearly to the margins of the area pellucida. Below the central part of the Scheibe is seen the loose mesenchymal mass of the preaxial or free mesoderm which is closely in contact with the entoderm and has begun to spread laterally (fig. 20). From this region there is a gradual transition to Hensen's node (fig. 21), characterized by the very thick pseudostratified ectoderm from the ventral surface of which cells are proliferating into a thick mass of mesoderm, consisting of loose

stellate cells extending to the entoderm. The pseudostratified ectoderm of the Scheibe here extends nearly to the margins of the area pellucida. The mesoderm is migrating laterally. At the posterior level of the node (fig. 22), the center of the streak shows a very shallow primitive groove, of the shape of a wide V. The central proliferating ectoderm and the mesoderm mass are slightly less thick here than at the anterior level of the node; but the mesoderm shows more extensive lateral migration, and has nearly reached the germ wall. It seems to migrate along the upper surface of the entoderm, which consists of a thin flattened membrane joining the germ wall laterally. Proceeding posteriorly (fig. 23), we find the groove more evident, the ectoderm of the streak less thick, and the mesodermal proliferation considerably diminished. The central ectoderm is still thickened and pseudostratified as the Scheibe, which, however, extends less than halfway to the margin of the area pellucida. The mesoderm forms a thin loose strip between ectoderm and entoderm, but is spread laterally throughout the whole area pellucida and has even penetrated into the area opaca. The entoderm is a thin membrane as before. At the extreme posterior end of the streak (fig. 24), the ectoderm is thin and has ceased proliferating mesoderm; there is no Scheibe. The mesoderm is a thin but dense layer and extends out over the germ wall. The entoderm is thicker and composed of two or three cell layers. There are irregular depressions in the ectoderm. The primitive groove continues to the posterior end of the streak.

The primitive streak of the duck can be divided into the same three regions emphasized by Wetzel for the chick: the anterior end with Hensen's node and the primitive pit; the flattened middle region, or Ebene, where the groove is shallow, and the posterior end, where the groove deepens again.

The germ wall has now for the first time become definitely organized into regions. At the margins of the area pellucida, it is protoplasmic, the protoplasm forming a network with large meshes, and with little yolk content. This may be called the germ wall proper, although it does not seem to be

cellular as stated by Lillie for the chick. It is continuous with the entoderm of the area pellucida. Lateral to this rather short protoplasmic region is a long zone of junction, composed also of a protoplasmic reticulum, but loaded with yolk, especially in its ventral part. It is overlaid by the very thin, membrane-like ectoderm. Outside of this is the margin of overgrowth composed of the membranous ectoderm above with a little yolky protoplasm beneath. This extends far out beyond the area pellucida. At the posterior end of the embryo the entoderm is continuous with the reticular protoplasm of the germ wall proper.

Measurements were made of various parts of the streak. The term streak here includes the ectoderm and the underlying attached mesoderm. Mesoderm cells which have become free and lie near the entoderm are not included. Through the anterior end of the streak the thickness varied from 40 to 72 μ, with an average of 56 μ. The width ranged from 80 to 200 μ; average, 136 μ. Through the posterior part of the streak, the thickness varied from 10 to 32 μ; average, 23 μ; the width from 64 to 240 μ; average, 154 μ. The streak is thus thicker anteriorly, but wider posteriorly. The ectoderm of the middle part of the Scheibe is 30 to 40 μ in thickness.

The distance of the anterior end of the streak from the anterior margin of the area pellucida diminishes during the development of the streak from an average of 1.35 μ in short streaks to 0.77 μ in the definitive stage. This indicates a forward progression of the streak, which is more marked than shown by the measurements, because meantime the area pellucida is expanding and thus moving away from the anterior end of the streak.

A study of the mitotic figures in thirteen definitive streaks, counted in the same manner as before, gave a total of 103 spindles at anterior levels, sixty-four in middle levels, and seventy-one near the posterior end. Again it is seen that the anterior end of the streak is the seat of the greatest proliferative activity. In the anterior end there were a total of forty-

four anteroposterior and forty-five lateral spindles. Through the middle the figures were twenty-four and forty-four, respectively, and posteriorly, twenty-nine and twenty-eight. This makes a total of 117 lateral spindles and ninety-seven anteroposterior spindles. As in previous stages, the majority of the spindles in anterior and middle levels lie in the sides rather than in the center of the streak. The results indicate postero-anterior and lateromedial migrations of cells.

HEAD-PROCESS STAGES

Head-process stages, preferably designated as primordial notochord stages, since the head process is the anlage of the notochord, not of the head, have been figured for many birds: for the chick by Duval and Wetzel, for the albatross, sparrow, and gannet by Schauinsland, and for the duck by Mitrophanow and Rabl. A total of thirty-two embryos were studied, fourteen as whole mounts, eighteen as sections. The area pellucida is much elongated, with an average length of 2.52 mm. The primitive streak, exclusive of the head process, averaged 1.32 mm., and has thus increased in length over definitive streak stages. The distance between the anterior margin of the area pellucida and Hensen's node has likewise lengthened to 1.2 mm., as compared with 0.77 mm. previously. The area pellucida has thus expanded, making it difficult to decide whether the streak has elongated in the anterior or posterior direction or both. It seems best to divide the head-process stage into two phases, early and late.

1. Early head process

According to Duval's atlas, this is reached in the chick by the nineteenth hour, with an area pellucida about 3.6 mm. long, primitive streak 1.7 mm., and head process 0.5 mm. These measurements were obtained from Duval's figure. In Rabl's four duck embryos of this time the area pellucida averaged 2.19 mm., the primitive streak 1.49 mm., and the head process 0.43 mm. in length. According to my findings, the head process first appears in the duck at about the

twenty-fourth hour. In thirty-two embryos the head process varied in length from 0.12 to 0.4 mm.; average, 0.20. Thus the duck requires longer to reach this stage than the chick and presents smaller measurements.

A duck embryo in early head-process stage is shown in figure 7. The area pellucida is elongated and pyriform, the primitive streak is in the definitive stage consisting of two parallel primitive folds, with the well-marked primitive groove between them. From Hensen's node a darkly stained short rod, the so-called head process, or primordium of the notochord, extends forward. In front of the head process is a transparent area devoid of mesoderm, and behind this a somewhat darkened area surrounding the head process and anterior half or two-thirds of the streak represents the Scheibe. The rear end of the streak is rather irregular and terminates in the germ wall.

Transverse and sagittal (fig. 25) sections of this stage show that the blastoderm anterior to the head process consists of a thin ectoderm and entoderm without mesoderm. Behind this the ectoderm over the head process thickens greatly into a pseudostratified epithelium with nuclei arranged in two or three layers. This is the primordium of the medullary plate, the former Scheibe. Below the anterior part of the medullary plate appears the scattered mesoderm of the prechordal plate (Adelmann, '22), thinner anteriorly, thicker posteriorly, and thinning again just in front of the head process. It extends some distance laterally in close contact with the entoderm. Behind the prechordal plate and continuous with it is the thicker, more compact mass of the chorda mesoderm (head process), eight to ten cells thick in front of the node, touching the medullary plate above, the entoderm below, passing posteriorly into the under surface of Hensen's node, and extending laterally as free mesoderm. The ectoderm of Hensen's node is a thick mass, which shows a slight depression tending to divide it into larger right than left portions; below there are still some indications of mesodermal proliferation. Behind the node the primitive pit forms a sharp

V-shaped depression in the very thick ectoderm of the Scheibe. Behind the pit, the streak consists of thick ectoderm two or three cells thick, which thins toward the posterior germ wall. Lateral to the anterior half or more of the streak the thickened ectoderm of the Scheibe extends to the margins of the area pellucida; but lateral to the posterior third of the streak, the ectoderm is scarcely thickened. Through middle levels of the streak the primitive groove is shallow and of thinner ectoderm; through posterior levels, its presence is barely indicated, but at the rear end it becomes deeper again and wider. The mesoderm is loose throughout the streak region, ten cells thick at the pit, thinning to three or four cells in thickness posteriorly. It extends laterally as thin loose sheets to the margins of the area pellucida at the pit level, in contact with the ectoderm out over the area opaca at more posterior levels. At the level of the posterior end of the streak, the mesoderm in the area opaca is greatly thickened into clumps preparatory to the formation of blood islands. The entoderm everywhere is a thin flat membrane, closely bound to the mesoderm of the head process and node, less in contact with it at middle levels of the streak and widely separated from the mesoderm at the rear end of the streak.

2. Late head process

The fully developed head-process stage is shown for the chick by Wetzel, for several birds by Schauinsland, and for the duck by Rabl. According to Duval's atlas, this stage is reached in twenty hours in the chick. Wetzel does not state the time definitely, but remarks that it occurs near the end of the first day. Rabl ('23) found it in the duck at twenty-nine to thirty hours; the average measurements of his three embryos are: length of the area pellucida, 2.65 mm.; length of the primitive streak, 1.7 mm.; length of the head process, 0.7 mm. I found that the duck embryo exhibits the maximum development of the head process at twenty-eight to thirty-one hours. Its length (determined from sections, since the structure is not clear in whole mounts) varied in twenty-one cases from 0.50 to 1.40 mm., with an average of 0.98.

The duck embryo at this time as seen in whole mounts (fig. 8) agrees very well with the drawings and descriptions of Wetzel (his fig. 32) for the chick and Rabl (his fig. 14) for the duck. The area pellucida is markedly pyriform. Behind the anterior transparent non-mesodermal area is the ridge marking the head fold with a darkly stained central part which represents the beginning of the brain. From this a paler band where the medullary folds arise extends back to the node. This whole nervous primordium is somewhat anchor-shaped. In it in late head-process stages the double ridge of the medullary folds, the right ridge always further developed and higher than the left, appears. The head process which lies beneath the medullary folds is scarcely visible in whole mounts except near the node. The node is a lens-shaped dark thickening cut in two longitudinally by a deep cleft, which passes through it asymmetrically so that the right part of the node is always larger than the left. Scheibe and streak are much as previously.

In studying the median sagittal section, the chief difference noted from the early head-process stage is that at the anterior end of the area pellucida, in the mesoderm-free region, the ectoderm and entoderm show a more marked elevation in what may now be termed the head of the embryo. The head fold or subcephalic pocket is indicated. In transverse sections of the head (fig. 26), the thickened elevated ectoderm of the future brain is underlaid by a thin entoderm on which rests in the median line the loose, laterally spread prechordal mesoderm. Through the level of the head process, the thickened medullary plate shows a central medullary groove, and right and left medullary folds, the right one higher and farther developed than the left. The head process is a compact mass below the medullary groove, to which it is strongly adherent, showing that it is notochord. Mesoderm extends lateral from the head process well out over the area opaca. The medullary groove becomes shallow over the posterior part of the head process and at the node passes into the primitive pit. The pit is a very deep cleft, almost touching

the entoderm below, slanted in such a manner that the right ectodermal fold of the node is higher and thicker than the left. Beneath the pit the mesoderm is scanty, but laterally it forms a thick layer extending out on the yolk and there thickening into blood islands. Posterior levels of late head-process stages are much like the earlier stage. The principal advance has been in anterior regions.

A neurenteric canal was noted by Balfour ('85, p. 163) in a duck embryo of twenty-six somites. I have not found any neurenteric canal in the stages studied, although the primitive pit in the late head-process stage is very deep, much deeper than that of chick embryos of corresponding degree of development. This fact at least suggests the possibility of the occurrence of a neurenteric canal in later stages.

The head-process stage is characterized in general by a decline of mesodermal proliferation from the streak and an increased activity of the ectoderm in forming the nervous system. The head process seems to arise as a forward migration of cells from the node. The greater activity of the right side of medullary plate and node is noteworthy.

HEAD-FOLD AND EARLY SOMITE STAGES

These stages are so well known in birds that only a few points will be mentioned. Rabl figured and described several duck embryos of these ages, which are in agreement with my observations. I have twenty-five whole mounts and twenty sectioned embryos belonging here. The head fold occurs in the duck around twenty-nine to thirty-three hours of incubation according to my and Rabl's findings, while in the chick as determined from Duval's plates, the time is about twenty-one hours. We may again consider the embryos in two stages.

1. Early head fold

This follows immediately upon the full development of the head process and differs from that stage chiefly by the formation of the head fold at the anterior end of the embryo. The head fold is caused rather by forward growth of the medul-

lary plate than by invagination of the proamnion—an explanation also given by Lillie for the chick. In whole mounts, the proamnion, the ridge of the head fold, its central darker brain thickening, and the medullary folds behind this are evident. In seventeen whole mounts of presomite head-fold stages, the right medullary fold was higher than the left in seven cases, while the two folds were approximately equal in the other ten. The medullary folds fade away toward the node. Node, pit, and streak are much the same as earlier. The posterior end of the streak is often irregularly sinuous and curved to one side. Of nineteen cases examined, nine were curved to the right, seven to the left, and three were straight. The Scheibe is still visible; blood islands appear around the posterior two-thirds of the area pellucida.

Sagittal sections show the head fold as a bay overhung by the thick anterior edge of the medullary plate, while the subcephalic pocket consists of the thin, widely separated ectoderm and entoderm of the proamnion. The anterior edge of the medullary plate is ten to sixteen cells thick and exhibits abundant mitoses indicating active proliferation. The prechordal mesoderm is proliferating anteriorly, as noted by Adelmann for the chick, and spreads laterally beneath the whole width of the medullary plate. In transverse sections are seen the well-developed medullary folds, equal or with the right one higher, the medullary groove with the round thick mass of notochord below, differentiated from the chorda mesoderm. The notochord joins the right and larger part of the node, while the medullary groove becomes continuous with the primitive pit. The streak still exhibits the three levels of Wetzel: anterior thick region with node and deep pit and groove; central Ebene with shallow groove, and posterior region where the groove is deeper again and the mesoderm thinner. The mesoderm is much the same as previously.

2. Early somite stage

The first somites appear in the chick at twenty-one hours, according to Duval; in the duck, as found by Rabl and me,

at about twenty-nine to thirty-five hours. I have three embryos showing the first intersomitic furrow, one incubated twenty-nine hours; two, thirty-two hours; six embryos with one somite, one of thirty hours, one of thirty-one hours, two of thirty-two and of thirty-four hours; nine with two somites, four of thirty-one hours, one each of thirty-two, thirty-four, and thirty-five hours, and two of thirty-three hours; and seven with three somites, three of thirty-three hours, two of thirty-one hours, and one each of thirty-five and thirty-six hours. The head fold deepens as the somites form and this relation is quite constant. The average diameter of the blastoderm was 9.31 mm.; length of the area pellucida, 3.21 mm. The primitive streak gradually decreases in length. In the head-process stage it averaged 1.32 mm. In the three embryos with the first intersomitic furrows it had decreased to an average of 1.3 mm. In the one-somite stage (six cases) it ranged from 1.2 to 1.4 mm., with an average of 1.28. At the two-somite stage (nine cases) the range was 1.0 to 1.4, with an average of 1.22 mm. For the seven three-somite specimens, the figures were 1.10 to 1.2 mm.; average, 1.13. The distance between Hensen's node and the anterior margin of the pellucid area is again increased, averaging 1.98 mm. This indicates either an elongation of the medullary-plate region or an expansion of the area pellucida.

As the anatomy of this stage presents nothing different from the well-known condition in the chick, a description of the sections is omitted.

RÉSUMÉ OF DEVELOPMENT

In the unincubated blastoderm of the duck, bilaterality has already been established as shown by a deeply stained crescentic area which is located at the future posterior end and is bisected by the later embryonic axis. This axis bears a relation which is approximately at right angles to the egg axis in slightly over 50 per cent of cases. This relation is preserved during incubation.

The unincubated blastoderm is composed of an outer, coherent, one-layered ectoderm and a lower incoherent entoderm, which is several layers thick posteriorly and thins anteriorly to a single layer. It consists mostly of separated cells. Encircling the area pellucida is the germ wall, much thicker at the future posterior end than elsewhere.

The diameter of the area pellucida and width of the area opaca remain unchanged during the first three hours of incubation, but thereafter increase at a gradually accelerating rate. The thick posterior germ wall decreases suddenly during the fourth to sixth hours to about three-fourths of its previous depth and remains in this state throughout the developmental period studied.

In the unincubated blastoderm the ectoderm of the posterior half of the area pellucida is slightly thickened as the precursor of the embryonic shield. This ectoderm increases in height during incubation and finally appears after several hours of incubation as the embryonic shield, which includes the ectoderm of the posterior half of the area pellucida. The primitive streak arises in the posterior part of the shield whose tall ectoderm becomes several-layered in the midline and begins to proliferate cells from its ventral surface. The streak is at first short and a little broad, but becomes longer and narrower until it attains the definitive stage. The primitive groove may appear at any stage of the streak, but it invariably is present only in the definitive stage; at first a shallow broad groove, it gradually deepens and narrows. Sections show that the primitive streak at all stages consists of a proliferating, thickened, several-layered ectoderm which occupies the whole length of the streak and reaches the germ wall posteriorly. The ectodermal thickening and proliferative activity are always greater at the anterior end of the streak, where at the definitive stage the thickening forms a definite mass, Hensen's node. The cells proliferated from the streak become the mesoderm. Study of mitotic figures shows that the greatest proliferative activity of the ectoderm is always at any stage in the anterior end of the streak (Hen-

sen's node) and diminishes posteriorly. The orientation of the spindles indicates postero-anterior and lateromedial migrations of cells. The elongation of the streak seems to be caused in part by more and more anterior ectoderm entering on proliferation and in part by migrations forward of cells that arose more posteriorly and migrations from the sides into the median line of cells that arose laterally.

The posterior part of the embryonic shield more or less disappears, but probably actually forms the rear part of the streak by migrating toward the midline. The anterior part of the original shield accompanies the anterior part of the streak as it grows forward and probably becomes the Scheibe or presumptive medullary plate. The Scheibe is a circular or oval area of very tall ectoderm which surrounds the anterior part of the streak. Later, as the time for the appearance of the medullary folds approaches, the Scheibe elongates and extends back about two-thirds of the length of the streak. The Scheibe is recognized by its very tall, pseudostratified appearance, with nuclei arranged in two or three strata.

Study of the number and orientation of mitotic figures in prestreak stages indicates that cells are moving from the rear part of the shield toward the midline and then moving forward in the midline. In the streak stages study of mitotic figures indicates that cells contribute to the middle regions of the streak by passing from the Scheibe toward the streak. The streak then elongates by the pushing apart of its ends through addition of cells to its central part.

The remainder of the Scheibe after these contributions to the streak is apparently all used up in the formation of the central nervous system. It seems to be drawn centrally as the medullary folds rise up and to be concentrated in them.

Hensen's node is a particularly thick and mitotically active part of the streak located at its anterior end. It is later cut asymmetrically by the deep primitive pit. Behind the pit the groove is shallow, then deepens again toward the rear end of the streak. The streak is thus divisible into three regions: anterior end, with Hensen's node and the deep pit;

middle region (Ebene of Wetzel), where the primitive groove is flat and shallow; and rear part, with a deeper groove. The primitive pit becomes continuous with the medullary groove and is always at the rear end of the latter.

The entire mesoderm arises from the lower side of the primitive streak along its whole length, apparently by actual proliferation of the ectoderm of the streak (see discussion). The mesoderm after it is formed begins to migrate laterally. This extension proceeds more rapidly at the posterior end of the streak and decreases anteriorly, so that the mesoderm always forms a pyriform area with the apex at the node. Migration laterally begins in the medium stages of the streak and progresses as the streak elongates. From the anterior and ventral side of the streak mesoderm extends forward in the midline as the free or preaxial mesoderm (prechordal plate). At the definitive streak stage the mesoderm at the node is thick, but does not extend very far laterally. Posteriorly it is thinner, but extends out beyond the margins of the area pellucida. In head-process stages the mesoderm extends at each side forward, so as to form horns enclosing the proamnion. The head process consists of mesoderm cells which migrate forward from the right side of Hensen's node and become the notochord.

The entoderm in the unincubated blastoderm is a loose layer of separate cells, several cells thick posteriorly, one layer thick throughout most of the area pellucida. It consists of separate rounded cells with many gaps between them, but underlies the whole area pellucida. Its posterior thickened part is continuous with the posterior thickening of the germ wall. Study of mitotic figures indicates that this posterior germ wall is the most active region of unincubated blastoderms and is adding cells into the entoderm. There was no evidence of the formation of the entoderm by an invagination, nor was any blastoporal split present; the observations indicate a formation of entoderm by forward proliferation from the posterior germ wall.

The entoderm becomes filled in to form a continuous coherent sheet during early incubation apparently by migration of cells from the thick posterior region of the entoderm. This process is complete just before the appearance of the primitive streak.

The entoderm is closely fused to the preaxial mesoderm, the head process, and Hensen's node, but is more or less separate from the mesoderm elsewhere.

There was no evidence of a definite Entodermhof or region of two-to-three-layered entoderm anterior to the primitive streak as described for the chick.

The entoderm continues everywhere into the germ wall, which is rather unorganized in early duck embryos, and cannot be differentiated into germ wall proper and zone of junction. This distinction appears first in the definitive streak stage, where the inner boundary of the germ wall becomes a protoplasmic reticulum, while its more peripheral portions remain yolk-laden. The germ wall throughout is thicker at the posterior end of the embryo than elsewhere and in early stages is here a region of mitotic activity.

At all of the stages studied the duck embryo is of smaller dimensions than chick embryos of the same degree of development and requires longer to attain the various stages than does the chick. The primitive streak in the duck begins at about the twelfth hour, the head process at the twenty-fourth hour, the first somites at twenty-nine to thirty-five hours.

DISCUSSION

1. Origin of the entoderm

Since in the unincubated blastoderm with which stage this study began the entoderm is already present, its origin was not definitely determined. The observations, however, are suggestive. The facts that the posterior germ wall is thickened and proliferating, that the entoderm is thick and many-layered in front of this, and thin anteriorly, and that the majority of the mitotic figures are oriented in an anteroposterior direction indicate that the entoderm is being formed by

the posterior germ wall and is pushed anteriorly by proliferation from behind. There was no evidence of a blastopore-like cleft in the posterior germ wall nor of an invagination process. The accepted theory of entoderm formation in birds by a gastrulation process at the posterior end of the blastoderm, in which the entoderm turns under and grows forward, rests almost entirely upon the observations of Patterson ('09) for the pigeon. My findings on the duck, while not consistent with Patterson's theory, do not lend any direct support to the existence of an actual invagination. They indicate a simple forward growth from a thickened posterior region.

2. Origin of the primitive streak

Numerous theories of the origin of the streak have been advanced, but practically all of the older ones are variations of the idea, which seems to have originated with Rauber ('76, p. 568, figs. 8, 9), that the streak is an elongated blastopore and is formed by the concrescence of the posterior margin of the blastoderm to either side of the middle line. In this category belongs the theory of Koller (pp. 186, 193) that the primitive streak arises by the forward growth of the central thickened part of a sickle-shaped ectodermal area (Sichel) found at the boundary of area pellucida and opaca at the posterior end of the blastoderm. Essentially similar is Duval's theory of the origin of the streak from the axial plate, a region at the rear end of the area pellucida where ectoderm is in contact with the underlying elements and in which the primitive streak arises by concrescence. The concrescence theory, probably based on erroneous observations, in part on abnormal embryos, has persisted to the present time as the chief theory of the formation of the streak. It was objected to by Mitrophanow ('02, p. 194), who claimed from a study of sections that in both chick and duck the ectodermal thickening which forms the streak arises near the center of the area pellucida and does not extend to the posterior germ wall. This does not agree with my findings for the duck nor Wetzel's for the chick. According to our observations, the primitive

streak appears morphologically as a slightly elongated thickening in the posterior part of the area pellucida and extends back to the posterior germ wall. The whole length of the streak is proliferating from the beginning. The streak is at first short and a little broad and extends about one-fifth the length of the area pellucida. The very broad hemispherical area depicted by Hoadley ('26), found by him in the chick at four hours' incubation, and considered by him to represent the earliest appearance of the primitive streak, is probably nothing but the embryonic shield. No other authors have figured such a streak in any birds, nor does the streak of the chick appear at such an early stage of incubation. The short streak consists of a many-layered ectoderm proliferating cells from its lower surface. The thickness of the ectoderm and its proliferative activity are greatest at the anterior end of the streak and decrease posteriorly, but both continue into the posterior germ wall.

Whether the streak actually arises in situ cannot be settled by purely morphological observations. The marking experiments of Wetzel on the chick indicate that in early blastoderms there is cell migration from the posterior lateral parts of the area pellucida toward the middle and along the midline from the posterior end forward. Gräper in his moving pictures of chick embryos of prestreak stages found a similar state of affairs. There was movement in the posterior part of the area pellucida from the lateral regions toward the middle and forward along the middle regions. Thus the streaming in the ectoderm has the form of a double whorl, backward along each side to the posterior end and forward along the middle. My data on the abundance and orientation of mitotic figures in prestreak duck embryos are in agreement with Gräper and Wetzel. The preponderance of anteroposterior and lateral spindles in posterior levels suggested cell movements from the sides toward the middle and from posterior regions forward along the midline. It thus appears that the cells of the short streak are not the same as those that earlier occupied this position, but have moved in from the sides.

Gräper states that the primitive streak is formed by these streaming movements, although it is not at all clear how he conceives this, unless he means that the cells heap up. The streak first appears in the center of the forward streams as a pit with sharp front and sides over which material streams down into the pit. It appears to me that neither Gräper nor Wetzel explains how the streak is formed by these movements.

After its first appearance the streak elongates and becomes somewhat narrower. We are here confronted with two questions. Does the streak elongate at its anterior or its posterior end or by its ends being shoved apart through elongation of the middle? And is the elongation the result of migration of the previously proliferating cells or do new levels of cells at anterior or posterior ends or both which were not previously proliferating begin to proliferate? The marking experiments of Wetzel have shown that marks placed in front of young streaks are moved forward and to the side, indicating a forward extension of the streak, in early stages. Marks at the rear show a backward movement at the time of the first elongation of the area pellucida. Thus early streaks are elongating at both ends. The elongation of the later streaks, however, is due chiefly to migration of cells from lateral regions into the middle part of the streak, pushing the two ends apart. Gräper states that at first the streak lengthens by the addition of material to its rear end through the streaming already described. Soon, however, the double whorl is replaced by lateromedial streaming. Material from the sides streams into the middle part of the streak, passes over the primitive folds down into the groove, and then turns and passes outward again as mesoderm. The results of Gräper and Wetzel therefore agree. My data on mitotic figures of early streaks indicate movements anteriorly in the streak and from the sides toward the middle; in definitive streak stages the greatest movements seemed to be from the sides into the middle.

Nevertheless, we do not get a clear picture of the growth of the streak. If the cells are continually moving, how do we

obtain a definite morphological picture? Is the ectoderm of the streak actually not proliferating at all, but only serving as a place where cells heap up and migrate down into the interior? Gräper seems to think this is the case. But my findings of abundant mitoses in the streak indicate actual cell proliferation, especially at the anterior end. It does not seem possible to answer the question whether hitherto inactive cells become involved in the streak activity and whether the original proliferating cells move into new situations. I am of the opinion that both processes occur. Morphological study shows merely that the band of apparently proliferating cells elongates.

From the work of Gräper and Wetzel and my own study it may be concluded that while it is true that cells are added to the streak from the sides, this does not occur in accordance with the concrescence theory. This theory of the formation of the streak is therefore incorrect, as far as the actual facts of present development are concerned. As to how the primitive-streak mode of development common to Amniotes may have evolved phylogenetically from the amphibian mode cannot be answered. In its physiological activity and rôle in development, the streak does seem to correspond to the dorsal lip of the amphibian blastopore (compare Wetzel, p. 280).

3. Formation of the node

Neither Gräper nor Wetzel presents any specific data on the formation of the node. Apparently this structure is merely a thicker and more actively proliferating portion of the streak, as shown by its appearance in section and the number of mitotic figures. It is not clear from Gräper's account of cell migration how this region happens to be thicker. One may suppose that in the forward migrations of cells they heap up in this region. Mitotic figures in my sections show clearly additions of cells to the median region by lateral mitoses. Sections of the node show more mitoses just at the sides of the node than in the node itself. The node may then represent a region of accumulation of cells moving in from behind and from the sides.

4. Origin of the head process

It seems evident from morphological study that the head process is simply a mass of mesoderm which migrates forward from the anterior and ventral surfaces of the node. This view is accepted in Lillie's text and is confirmed by Wetzel's marking experiments. It cannot be formed by a backward moving of the node, leaving the head process in front of it, because at this time the streak is at its maximum length and shows no evidence of any shortening. The backward progression of the node does not begin until after the head process has been fully formed.

5. Later stages

Direct observations together with the moving pictures of Gräper and the marking experiments of Wetzel leave no doubt that after the head-process stage, the node moves rapidly backward until the somites are completed. This is evidenced in my study by the progressive shortening of the primitive streak and lengthening of the medullary-plate region. The rear end of the nervous system is always at the primitive pit. As the node moves back it deposits the notochord in front of it. The notochord can always in sections be traced into the node, with which it remains continuous during its formation. It seems rather strange that up to the last few years this rather obvious process of backward retreat of the node should have escaped the attention of embryologists.

Since the results have been summarized in a résumé preceding the discussion, a formal summary is omitted.

LITERATURE CITED

Adelmann, H. B. 1922 The significance of the prechordal plate: An interpretative study. Am. J. Anat., vol. 31, pp. 55–101.

v. Baer, C. E. 1828 Ueber die Entwickelungsgeschichte der Thiere. Beobachtung und Reflexion. I. Theil, p. 12.

Balfour, F. M. 1885 Balfour, Francis Maitland, Complete Work. Memorial Edition, vol. 3, p. 163.

Bartelmez, G. W. 1918 The relation of the embryo to the principal axis of symmetry in the bird's egg. Biol. Bull., vol. 35, pp. 319–361.

DUVAL, M. 1884 De la formation du blastoderme dans l'oeuf d'oiseau. Ann. des Sc. Nat. Zool., T. 18, pp. 1–208.

———— 1889 Atlas d'embryologie, pp. 27–38.

DALTON, J. C. 1881 The position of the embryo in the fowl's egg. Med. Record, vol. 20, p. 589.

EDWARDS, C. L. 1902 The physiological zero and the index of the development for the egg of the domestic fowl, Gallus domesticus. Am. J. Physiol., vol. 6, pp. 351–397.

FÉRÉ, CH. 1896 Note sur l'orientation et sur l'allure du développement de l'embryon de canard. C. R. Soc. Biol., T. 48, pp. 909–910.

———— 1897 Note sur le développement et la position de l'embryon du poulet dans les oeufs à deux jaunes. C. R. Soc. Biol., T. 4, sér. 10, pp. 858–860.

GRÄPER, L. 1911 Beobachtung von Wachstumsvorgängen an Reihenaufnahmen lebender Hühnerembryonen nebst Bemerkungen über vitale Färbung. Arch. f. Entwmech., Bd. 33, S. 303–327.

———— 1926 Die frühe Entwicklung des Hühnchens nach Kinoaufnahmen des lebenden Embryo. Anat. Anz., Bd. 61, Ergänzungsheft, S. 54–58.

———— 1929 Die Methodik der stereokinematographischen Untersuchung des lebenden vitalgefärbten Hühnerembryos. Arch. f. Entw., Bd. 115, S. 523–541.

———— 1929 Die Primitiventwicklung des Hühnchens nach stereokinematographischen Untersuchungen, Kontrolliert durch vitale Farbmarkierung und vergleichen mit der Entwicklung anderer Wirbeltiere. Arch. f. Entw., Bd. 116, S. 382–429.

HASWELL, W. A. 1887 Observations on the early stages in the development of the emu (Dromaeus novae hollandiae). Proc. Linn. Soc. New South Wales, ser. 2, pp. 577–600.

HIS, W. 1868 Untersuchungen über die erste Anlage des Wirbelthierleibes. Die erste Entwickelung des Hühnchens im Ei, S. 1–97. Leipzig.

HOADLEY, L. 1926 Developmental potencies of parts of the early blastoderm of the chick. II. The epidermis and feather primordia. J. Exp. Zoöl., vol. 43, p. 183.

HYMAN, L. H. 1927 The metabolic gradients of vertebrate embryos. III. The chick. Biol. Bull., vol. 52, pp. 1–38.

KOLLER, C. 1882 Untersuchungen über die Blätterbildung im Hühnerkeim. Arch. f. mikr. Anat., Bd. 20, S. 174–211.

KOPSCH, F. 1927 Die Lage der Primitivstreifens in Hühnerei. Zeit. f. mikr. Anat., Bd. 8, S. 185–192.

LILLIE, F. R. 1919 The development of the chick. Henry Holt & Co.

MITROPHANOW, P. 1899 Beobachtungen über die erste Entwickelung der Vögel. Anat. Hefte, Bd. 12, S. 155–297.

———— 1902 Beiträge zur Entwicklung der Wasservögel. Zeit. f. wiss. Zool., Bd. 71, S. 189–210.

PATTERSON, J. T. 1909 Gastrulation in the pigeon's egg. A morphological and experimental study. J. Morph., vol. 20, pp. 65–123.

RABAUD, E. 1908 La position et l'orientation de l'embryon de poule sur le jaune. Arch. Zool. Ex. Gén., sér. 4, T. 9, Notes et Revue No. 1, pp. i–vi.

RABL, C. 1923 Ueber den Primitivestreifen und Kopfortsatz der Ente. Morph. Jahr., Bd. 52, S. 111–142.

RAUBER, A. 1876 Primitivrinne und Urmund. Beitrag zur Entwicklungsgeschichte des Hühnchens. Morph. Jahr., Bd. 2, S. 550–576.

SCHAUINSLAND, H. 1903 Beiträge zur Entwickelungsgeschichte und Anatomie der Wirbeltiere. II. Studien zur Entwickelungsgeschichte der Sauropsiden. Zoologica, Bd. 16, S. 101–133.

WALDEYER, W. 1869 Bemerkungen über die Keimblätter und den Primitivstreifen bei der Entwicklung des Hühnerembryo. Zeit. f. rationel. Med., Bd. 34, S. 159–178.

WETZEL, R. 1929 Untersuchungen am Hühnchen. Die Entwicklung des Keims während der ersten beiden Bruttage. Arch. f. Entw., Bd. 119, S. 188–321.

ABBREVIATIONS

a, anterior end of blastoderm
ec, ectoderm
en, entoderm
gw, germ wall
hp, head process
m, margin of overgrowth
me, mesoderm
mp, medullary plate
n, Hensen's node
p, posterior end of blastoderm
pf, primitive fold
pg, primitive groove
pm, preaxial mesoderm
pp, prechordal plate
ps, primitive streak
pt, primitive pit
s, Scheibe
sh, embryonic shield

The drawings of plates 3 and 4 were made at an initial magnification of 100 diameters and reduced to 50 diameters in reproduction.

PLATE 1

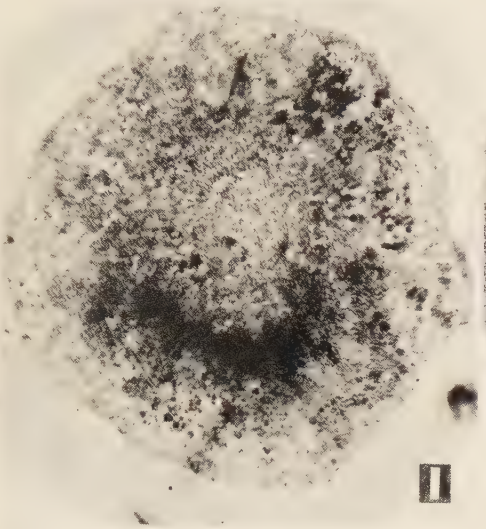
EXPLANATION OF FIGURES

1 Photograph of an unincubated blastoderm (41-1), showing the posterior thickened germ wall. × 28.

2 Photograph of a blastoderm in the shield stage (17-11). Age, eleven hours. × 28.

3 Photograph of a blastoderm in the short primitive-streak stage (84-11). Age, fourteen hours. × 18.

4 Photograph of a blastoderm in the medium primitive-streak stage (12-18). Age, sixteen hours. × 23.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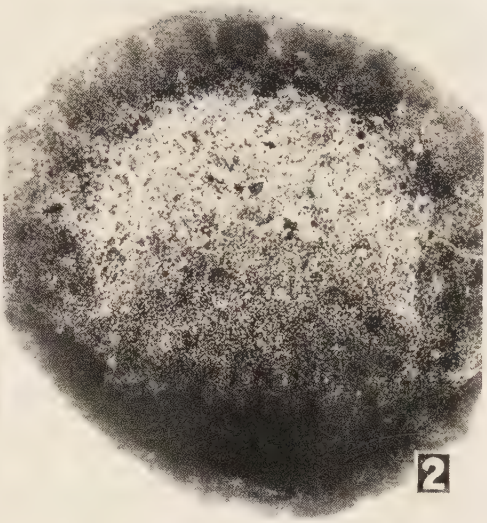
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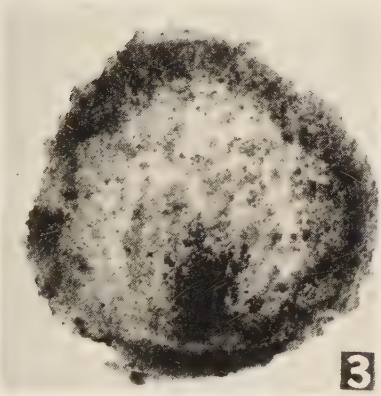
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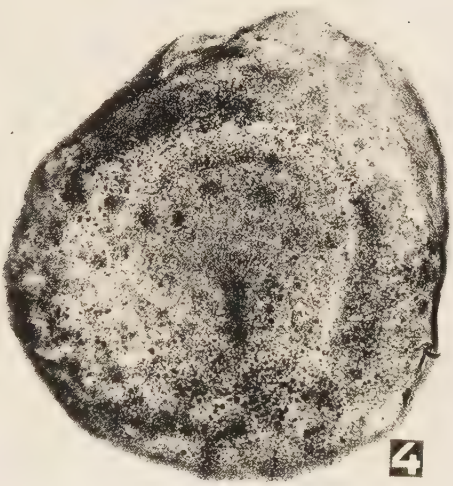
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PLATE 2

EXPLANATION OF FIGURES

5 Photograph of a blastoderm in the elongated primitive-streak stage (14-19). Age, nineteen hours. × 23.

6 Photograph of a blastoderm in the definitive primitive-streak stage (10-25). Age, twenty-four hours. × 26.

7 Photograph of a blastoderm in the head-process stage (16-27). Age, twenty-eight hours. × 20.

8 Photograph of a blastoderm (35-31) in medullary-plate stage. Note anchor-shaped medullary plate extending from right half of the node. Age, thirty-one hours. × 20.

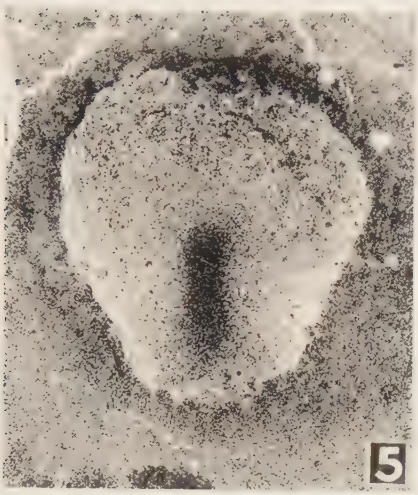
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PLATE 3

EXPLANATION OF FIGURES

9 Median sagittal section of an unincubated blastoderm (26-1). Note especially the thickened posterior germ wall (compare fig. 1).

10 Median sagittal section of a blastoderm in the shield stage (9-8), corresponding to stage shown in figure 2. Age, ten hours. Note embryonic shield and its relation to the thickened germ wall, the extent of the margin of overgrowth, and that anteriorly the entodermal cells are not yet formed into a coherent layer.

11 and 12 Transverse sections through a blastoderm in the short primitive-streak stage (4-13), corresponding to stage shown in figure 3. Age, twelve hours.

11 Through the area pellucida anterior to the streak.

12 Through the middle level of the streak.

13 to 15 Transverse sections through a blastoderm in the medium primitive-streak stage (3-31), corresponding to stage illustrated in figure 4. Age, seventeen hours.

13 Through a level of the area pellucida anterior to the streak.

14 Through a level of the anterior end of the streak. Note slight amount of mesoderm below streak.

15 Through the posterior end of the streak.

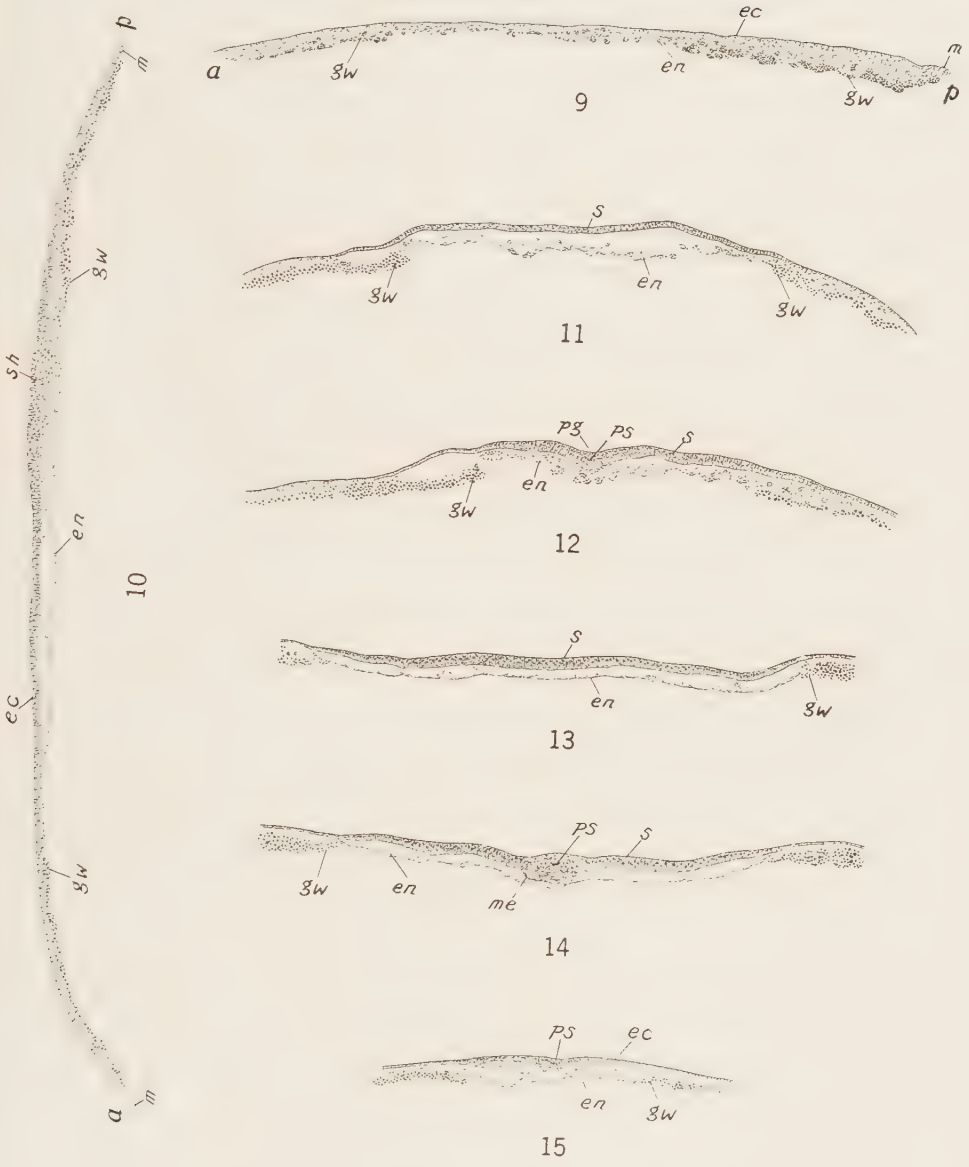
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PLATE 4

EXPLANATION OF FIGURES

16 to 19 Transverse sections through a blastoderm in the elongated primitive-streak stage (36-13), corresponding to the stage shown in figure 5. Age, twenty hours.

16 Through the area pellucida anterior to the streak. Note Scheibe and preaxial mesoderm.

17 Through the anterior end of the streak.

18 Through the posterior end of the streak.

19 Through the extreme posterior end of the streak above the germ wall.

20 to 24 Transverse sections through a blastoderm in the definitive streak stage (10-21), corresponding to stage shown in figure 6. Age, twenty-three hours.

20 Through level of the area pellucida anterior to the streak.

21 Through level of Hensen's node.

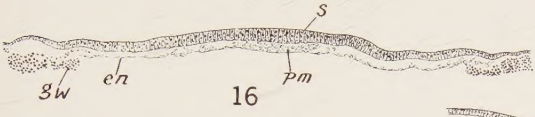
22 Through the posterior level of the node.

23 Through the middle level of the streak.

24 Through the posterior end of the streak.

25 Median sagittal section of a blastoderm in the head-process stage (4-23), corresponding to stage shown in figure 7. Age, twenty-seven hours.

26 Transverse section through the anterior end of the medullary plate of a blastoderm in the late head-process stage (15-32), corresponding to stage shown in figure 8. Note especially the thickened medullary plate and the prechordal plate. Age, twenty-nine hours.



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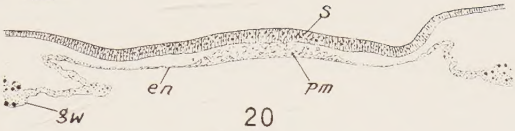

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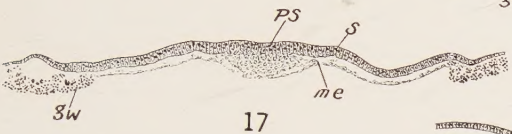

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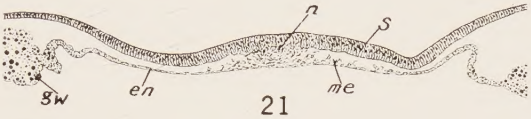

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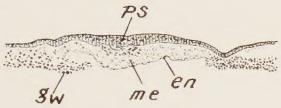

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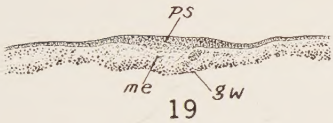

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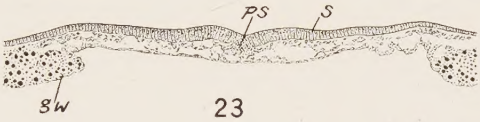

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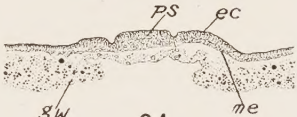

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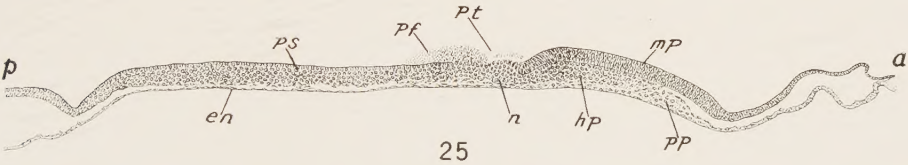

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